

FIG. 1

Context-Sensitive Parallel Optimization

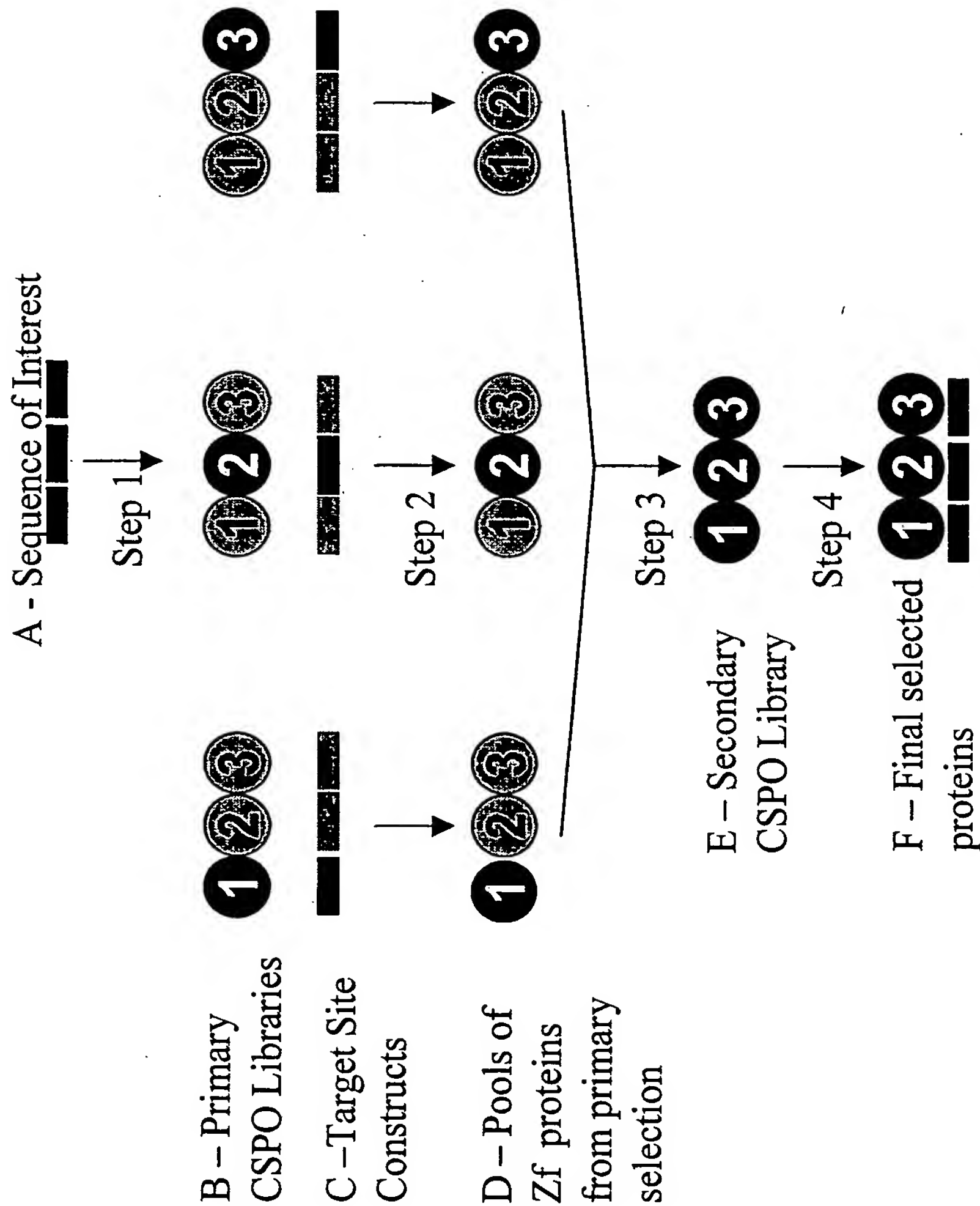


FIG. 2

Construction of Randomly Recombined Libraries

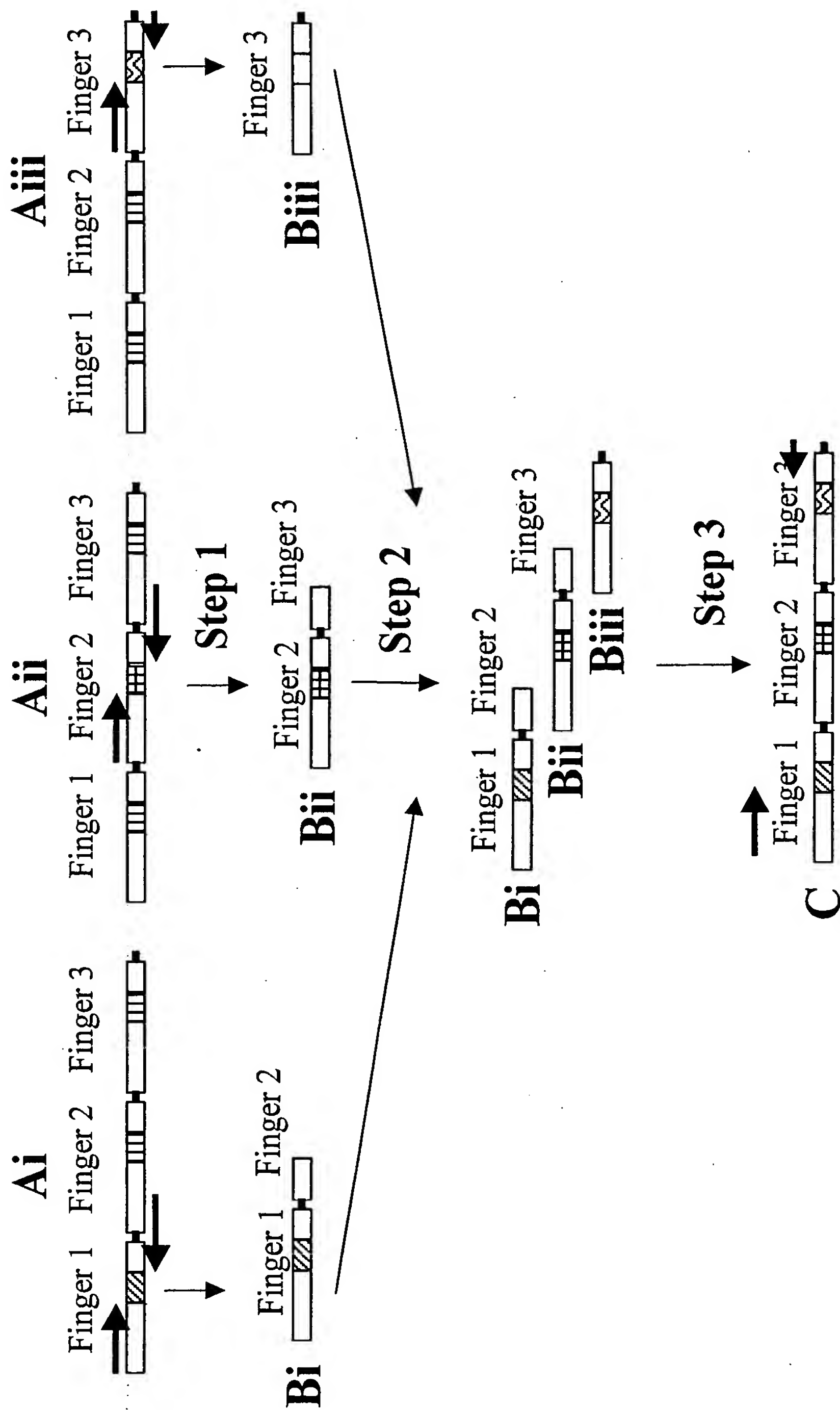


FIG. 3
Quantifying Affinity of ZFPs

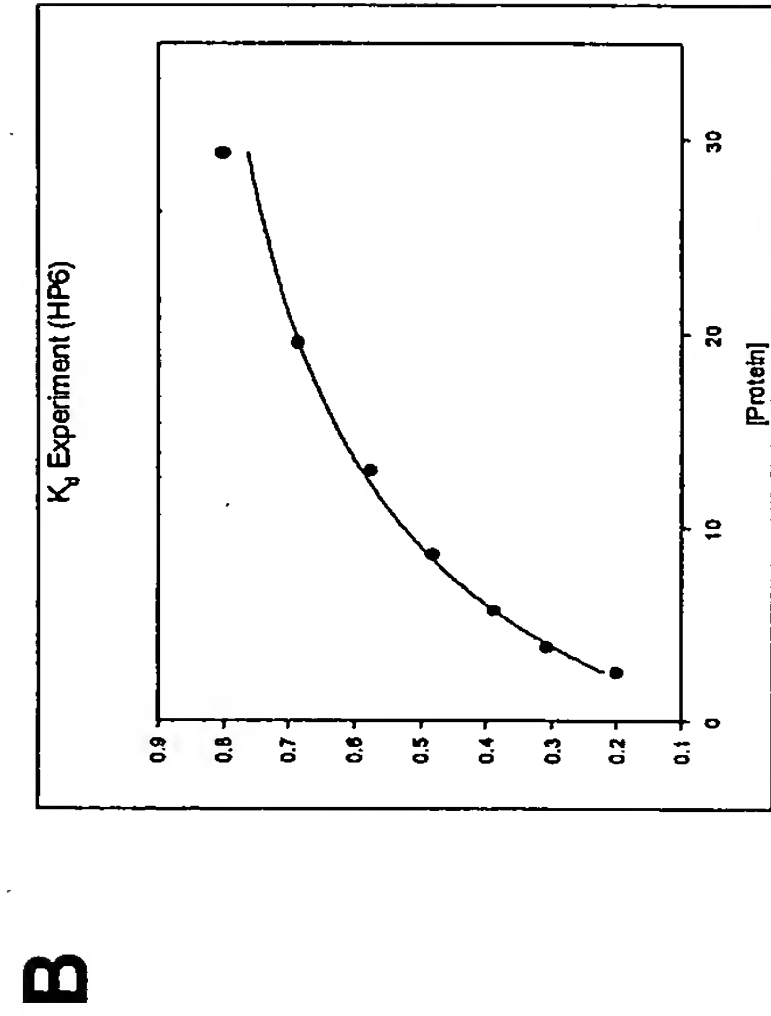
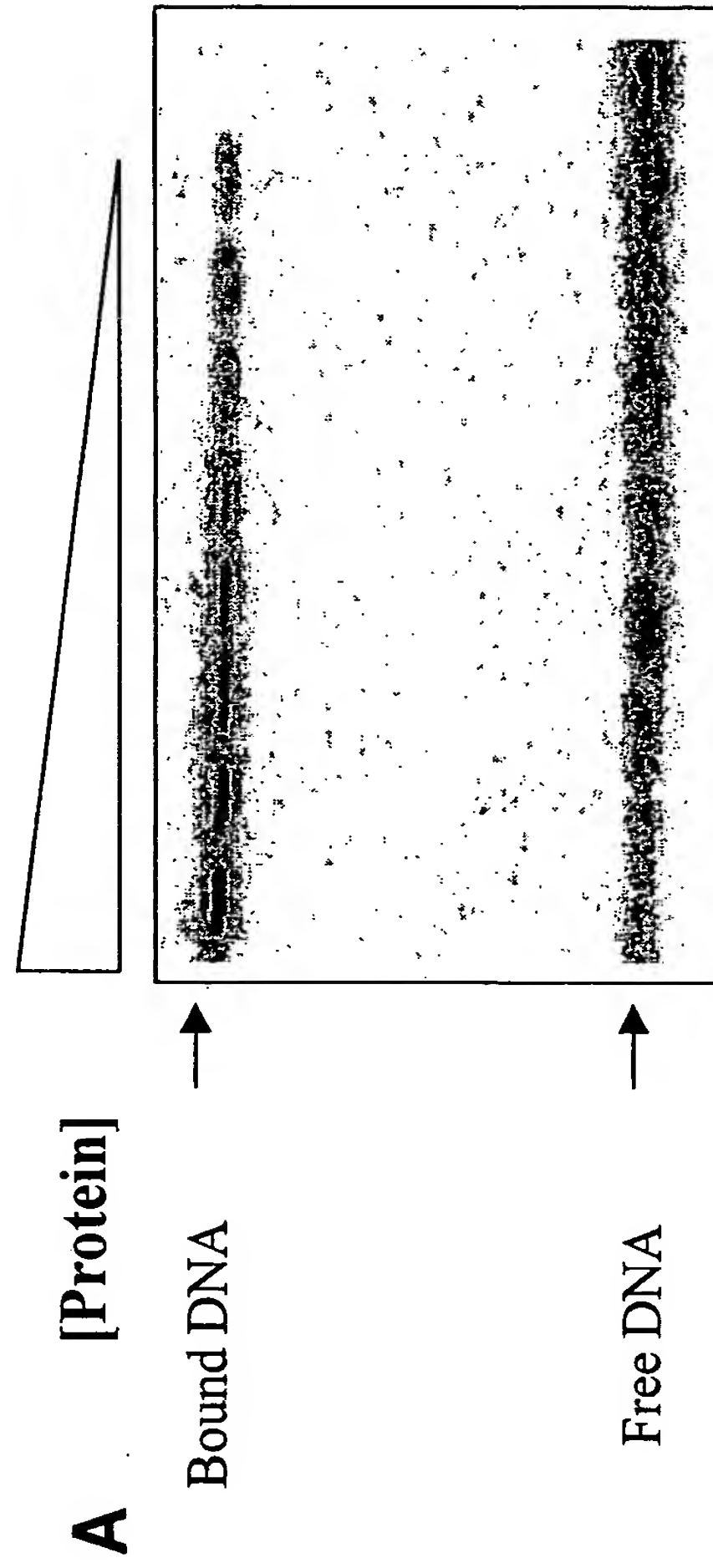


FIG. 4
Characterizing Specificity of ZFPs

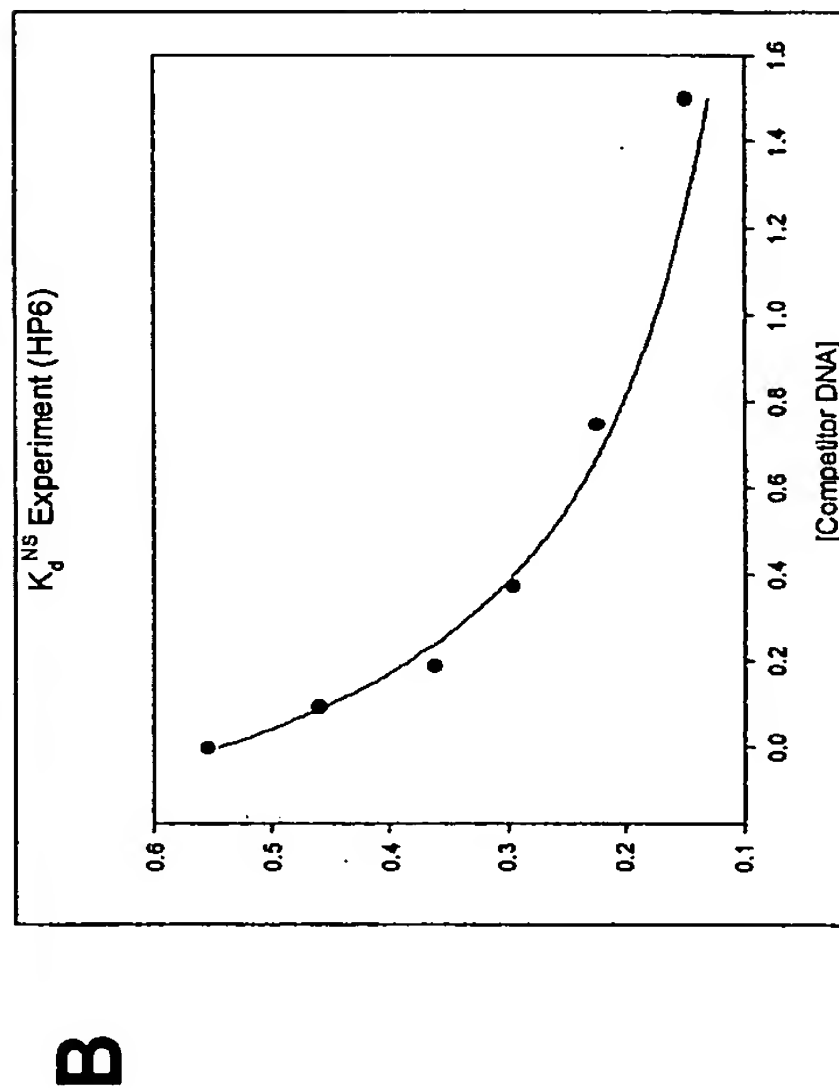
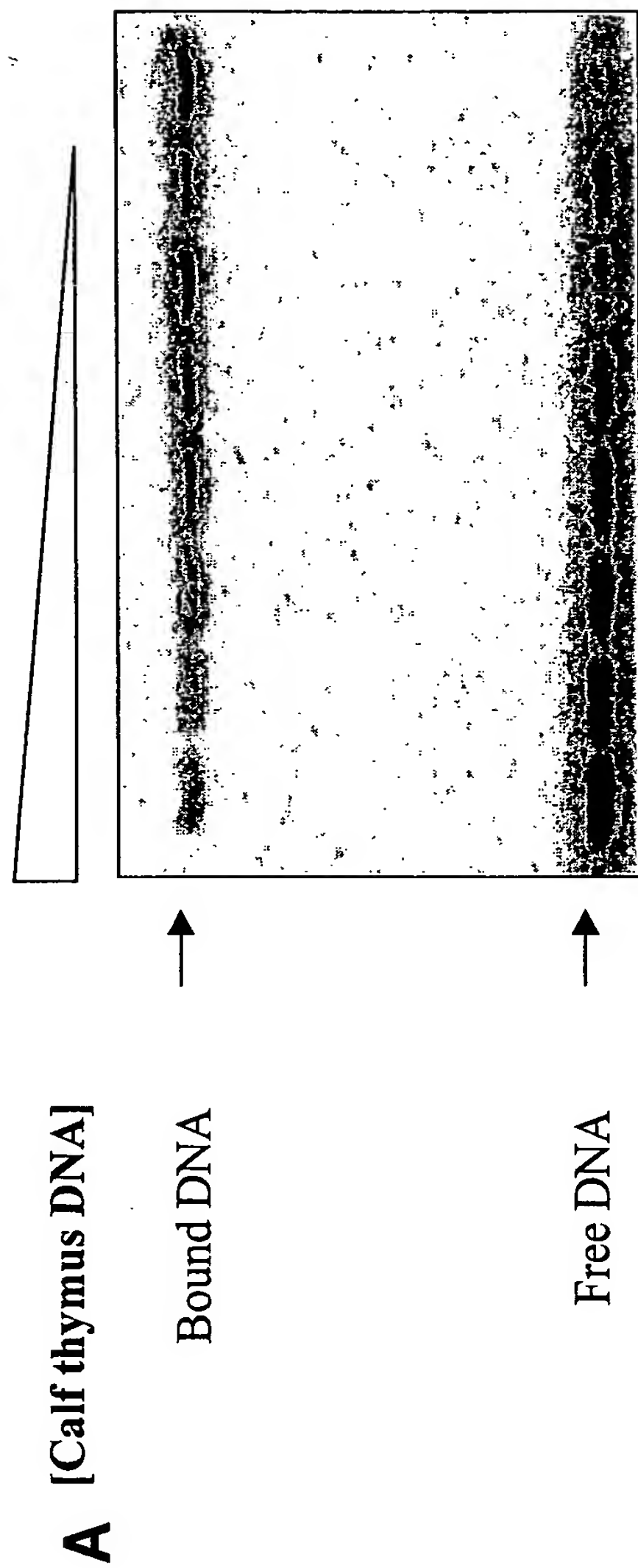


FIG. 6
Selections for the BCR-ABL site

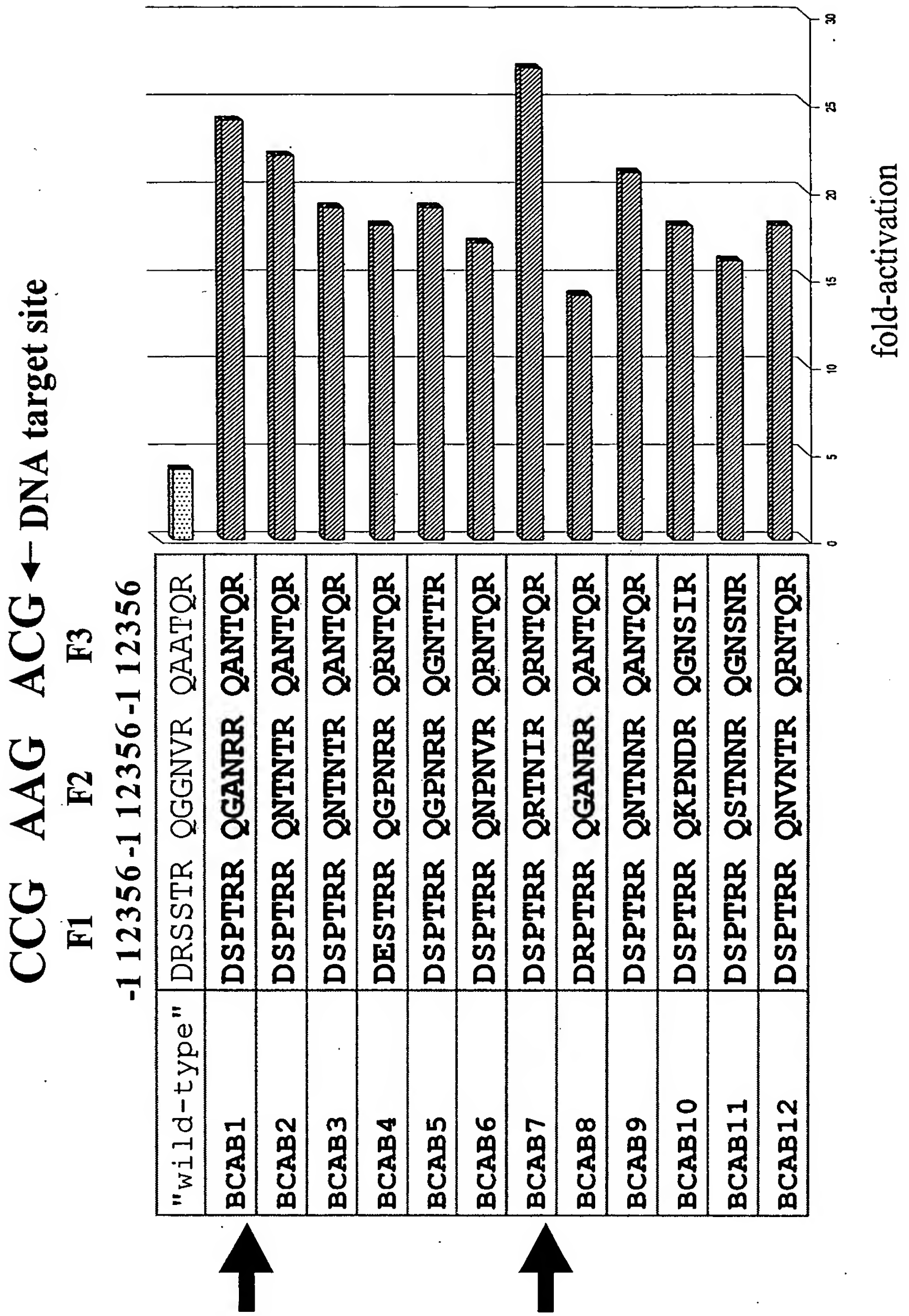


FIG. 7

In vitro characterization of BCR-ABL ZFPs

| Protein | Sequence | K_d^{spec} (pM) | $K_d^{non-spec}$ (nM) | Specificity ratio | # of DNA bases specified |
|---------|-----------------------|----------------------|--------------------------|----------------------|--------------------------------|
| "wt" | DRSSTR QGGNVR QAAATQR | 28 (± 3.9) | 55 (± 12) | 1,980 | ~5.5 |
| BCAB 1 | DSPTRR QGANRR QANTQR | 78 (± 13) | 2100 (± 270) | 27,000 | ~7.4 |
| BCAB 7 | DSPTRR QRTNIR QRNTQR | 60 (± 8.5) | 1300 (± 97) | 23,000 | ~7.2 |
| Zif268 | | 8.1 (± 1.8) | 1000 (± 120) | 130,000 | ~8.5 |

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FIG. 8

Selections for the erb-B2 site

GTG ACG CCG ← DNA target site

F1 F2 F3

-1 12356 -1 12356 -1 12356

| | | | |
|-------------|--------|--------|--------|
| "wild-type" | RKDSVR | QSGDRR | DCRDAR |
| EB1 | RQDIVK | QSSTTR | EKQGHR |
| EB2 | RSDVAN | QSSTTR | ERQGKR |
| EB3 | RSDVAN | QSSTTR | ERQGKR |
| EB4 | RSDLRK | QSSTTR | ERQGKR |
| EB5 | RPDVVK | QSSTTR | DSTTRR |
| EB6 | RSDVAN | QSSTTR | EKQGGR |
| EB7 | RSDLTK | QSGTKR | ERQGKR |
| EB8 | RSDLTK | QSSTTR | EKQGRR |
| EB9 | RSDVSK | QSTTKR | ERQGKR |
| EB10 | RQDIVK | QSSTTR | EKQGHR |
| EB11 | RSDLTK | QSSTTR | ERQGKR |
| EB12 | RPDVQK | QSGTVR | DQSNRR |

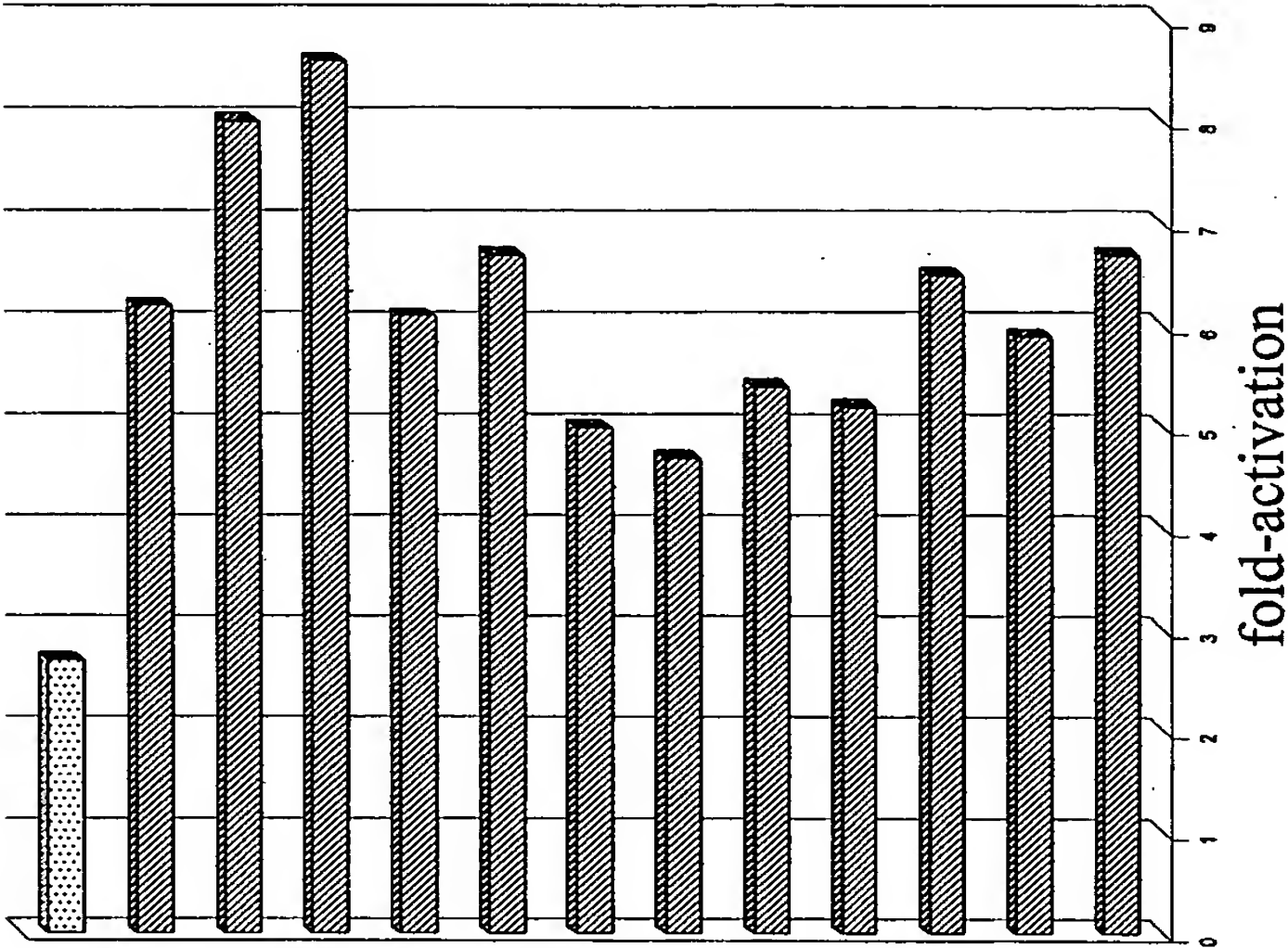


FIG. 9

In vitro characterization of erb-B2 ZFPs

| Protein | Sequence | K_d^{spec} (pM) | $K_d^{non-spec}$ (nM) | Specificity ratio | # of DNA bases specified |
|---------|----------------------|----------------------|--------------------------|-------------------|--------------------------|
| "wt" | RKDSVR QSGDRR DCRDAR | 150 (± 23) | 1000 (± 120) | 6,700 | ~6.4 |
| EB 3 | RSDVAN QSSSTR ERQGKR | 31 (± 3.1) | 1100 (± 15) | 35,000 | ~7.5 |
| EB 11 | RSDLTK QSSSTR ERQGKR | 65 (± 3.9) | 1100 (± 81) | 17,000 | ~7.0 |
| Zif268 | | 8.1 (± 1.8) | 1000 (± 120) | 130,000 | ~8.5 |

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FIG. 10

Selections for the HIV promoter site

ACG TCG TAG ← DNA target site
F1 F2 F3

-1 12356-1 12356-1 12356

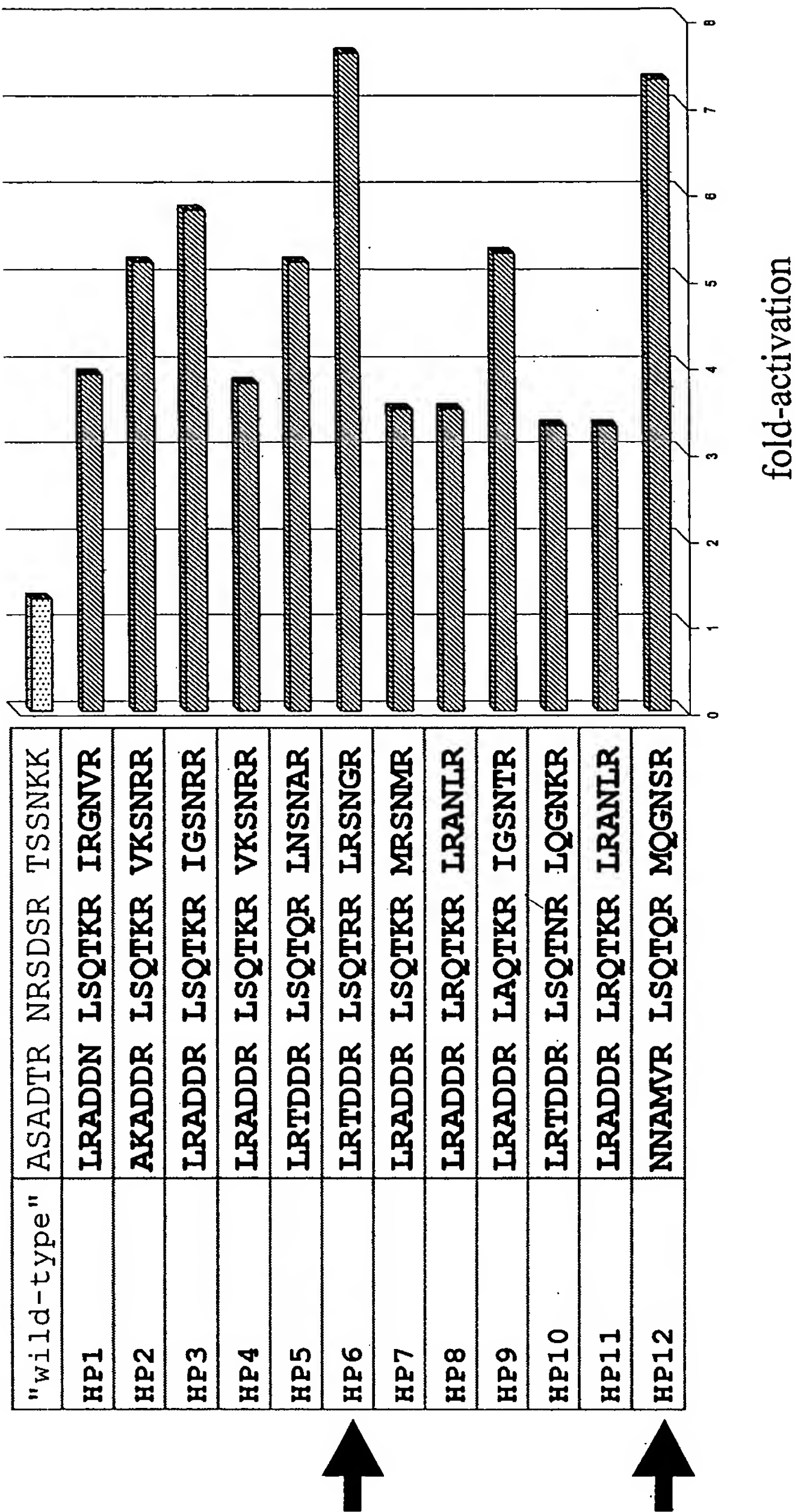


FIG. 11

In vitro characterization of HIV Promoter ZFPs

| Protein | Sequence | K_d^{spec} (pM) | $K_d^{non-spec}$ (nM) | Specificity ratio | # of DNA bases specified |
|---------|----------------------|--|--------------------------|----------------------|--------------------------------|
| "wt" | ASADTR NRSDSR TSSNKK | Unable to calculate (does not bind in vitro) | | | |
| HP6 | LRTDDR LSQTRR LRSNGR | 9.3 (± 1.2) | 820 (± 74) | 87,000 | ~8.2 |
| HP12 | NNAMVR LSQTQR MQGNSR | 9.3 (± 0.39) | 180 (± 8.8) | 19,000 | ~7.1 |
| Zif268 | | 8.1 (± 1.8) | 1000 (± 120) | 130,000 | ~8.5 |

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